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NO DRAWINGS

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COMPLETE SPECIFICATION

Improvements in or relating to Additives for Bread and other Fermented Products

We, WILLIAM LEONARD RUSSELL and WILFRED JOHN RUSSELL, British Subjects, of Albert Works, Chatsworth Road, Stratford, London, E.15, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with additives for bread and other leavened bakery and with methods of making such bakery when using these additives.

It is an object of the present invention to produce a bread additive which enhances the leavening of the bread and which, at the same time, minimizes the rate at which the bread becomes stale. Anti-staling additives are particularly desirable when making loaves which are subsequently to be sold as a sliced bread.

According to one aspect of the present invention, an additive for bread or other leavened bakery consists of, or includes, at least one fat or oil, and at least one proteolytic enzyme derived from Bacillus subtilis or Aspergillus oryzae.

According to a second aspect of the present invention, in a method of making bread and other leavened bakery, an additive consisting of, or including, at least one fat or oil, and at least one proteolytic enzyme derived from Bacillus subtilis or Aspergillus oryzae is added to flour or dough before it is allowed to ferment.

It is preferred that the additive should also contain at least one phospholipid or phospholipid containing material. Suitable examples of phospholipid containing materials are soya flour and wheat germ oil. In particular, it is desirable to use comminuted soya bean which is frequently known as "full fat" soya flour.

Full fat soya flour has a phospholipid content of up to 21%. The amounts of soya flour which may be used may vary between 4 oz. and 10 lbs. per sack of flour of 280 lbs. At lower concentrations the effect of the soya flour will be very small, whilst at higher concentrations a distinct flavour and a creamy colour will be apparent in the finished bread. Usually the phospholipid-containing material should be present in the additive in a proportion of between 15% and 95% by weight.

Fats or oils employed in accordance with the present additive may be natural or animal fats such as lard, compound vegetable fats prepared by selective blending and hydrogenation of vegetable fats or oils, or partially esterified polyhydric alcohols, with or without tartryl or ethylene groups. It is preferred to use super glycerinated fats, a particularly preferred fat being commercial glyceryl monostearate which may contain approximately 35% to 45% of the monostearate, together with di- and tristearates, and up to 5% of one or more soaps. Preferably the fat should be plastic at room temperatures, so that during the preparation of the additive it will become adsorbed on to soya flour incorporated therein, and will form an intimate mixture with it. The fat may be rendered plastic during manufacture by the incorporation of a proportion of free glycerol, or by suitable machining. Preferred fats are those which are obtainable in a finely powdered form and which are so processed as to be suitable for use without prior emulsification.

The proteolytic enzyme derived from Bacillus subtilis or Aspergillus oryzae may suitably be formed by the method described in British Patent Specification No. 701,191. The enzyme may suitably be employed as a fine powder mixed with a maize starch diluent and stabilised by means of a suitable antiseptic. The concentration of the enzyme in the additive should be adjusted to give the additive the required proteolytic activity.

The proteolytic activity of the enzyme may be measured in "proteinase units", 10 proteinase units being the quantity of enzyme which will solubilize 8.33 mg. of nitrogen from a 3% aqueous solution of casein at pH 6.5 and a temperature of 35°C. in thirty minutes, that is to say, which will during this period convert 8.33 mg. of the nitrogen content of the casein into a soluble form which can be estimated by the Kjeldahl method. For convenience a further unit, the Xn unit, is defined as being equal to 20 proteinase units.

The enzyme preparation may be adjusted to any desired concentration, Bacillus subtilis enzyme being generally employed at a concentration of 18 Xn units per gram, while Aspergillus oryzae enzyme may be employed at a concentration of a 4 Xn units per gram.

Whatever the concentration or type of the enzyme, the proportion incorporated into the additive should be such that the proteolytic activity of the additive will lie between 0.02 and 0.2 Xn units per gram, preferably 0.07 Xn units per gram. Thus, when an enzyme preparation having an activity of 18 Xn units per gram is employed, it should be incorporated into the additive in a proportion of 0.39% by weight.

The performance of the present invention is not dependent on theoretical considerations, and this Specification should not be so construed. However, it is believed that the action of the additive according to the present invention is as follows. First, the additive causes a definite modification of the gluten structure during the fermentation process so that the dough is rendered more extensible, easier to work and less likely to damage during the manipulation and machining necessary in bread production. This effect can be discerned by the production of bread which has a softer crumb and finer texture than is the case in conventional bread without loss of crumb stability.

Secondly, gas production is stimulated throughout the leavening process and especially during the later and more important stages of "final proof". It has been experimentally shown that the "maltose figure" of a flour may be increased from 1.87 to 2.10% by weight (Amended Rumsey) by the addition of 0.003% of proteolytic enzyme having an activity of 18 Xn units per gram.

These figures compare favourably with those obtained when diastatic malt flour is added to the flour at 0.3% of the flour weight if it is realised that this latter additive is prepared specifically for its diastatic activity. Experiments have shown that amylolytic preparations of fungal origin have little or no effect on the maltose figure of a flour, and it would therefore appear that the proteolytic enzyme subjects the starch cells in the flour to degradation by proteolysis. It is thought that this effect may be brought about by a splitting off of protein-bound amylases from the starch cell.

The increase in diastatic as well as proteolytic activity brought about by the proteolytic enzyme preparation indicates that greater supplies of carbohydrate foods become available to the yeast used in the fermentation process. It is desirable, therefore, to incorporate a source of amino nitrogen into the present additive so as to improve the metabolism of the yeast organism and thus to enable the yeast to assimilate the extra sugars. As fermentation proceeds, ample supplies of simple protein foods will become available in the normal course of the process, supplemented in this instance by the breakdown products due to the action of the added proteolytic enzyme. We have found by experiment that ammonium chloride is a suitable source of amino nitrogen, although other ammonium salts may be used, and the preferred proportion of the ammonium chloride is 6.25% of the additive. This proportion may be varied between 2% and 12% according to current requirements dictated by variations in the flour, yeast or other bakery materials.

The calcium ion plays an important part in the leavening process. It is thought to stabilise amylase activity and acts as an important "trace" element in the metabolism of the yeast organism. While in "hard" water districts, sufficient amounts of this element will generally become available from the water used in doughmaking, in "soft" water districts this may not be the case. Accordingly, a proportion of calcium sulphate is normally included in the present additive, although other calcium salts may be used. The preferred proportion is 12.10% although this proportion may be varied between 4% and 20% according to the requirements dictated by variations in flour, water, yeast and other bakery materials.

It has been found that the presence of bromates in the present additive adversely effects the performance of the enzyme. When, therefore, this Specification refers to the use of ammonium or calcium salts, the reference should be construed as excluding the bromates.

The action of the fat or oil contained in the present additive on the flour is thought to be as follows. It is believed that water in the dough becomes physically bound to the flour lipids. The binding of the water is accompanied by a decrease of the swelling power of the starch in the flour and a retardation of the release of water from the soluble starch (amylose) fraction of this starch. When the flour is wetted during the manufacture of dough, a protein lipid complex is thought to be formed, which complex binds in the lipid fraction. Added fatty esters, particularly super glycerinated esters which have a high percentage of hydrophilic groups,

tend to bind water to this complex so that the protein becomes more completely hydrated and the diffusion of water from the baked bread product becomes restricted. It is possible to add up to three times the original amount of lipid in the flour in order to enhance this effect. Phospholipids such as those in comminuted soya flour have been found to be particularly effective for this purpose. It has been experimentally shown that the soya flour-ester mixture has a softening effect on the crumb of the bread, this effect being thought to be partly caused by the retention of water in the dough caused by the addition of this mixture. This effect appears to be greatly enhanced by the addition of the proteolytic enzyme used in the present invention.

An additive according to the present invention may suitably be prepared in the following manner. A horizontal mixer, fitted with a worm agitator and an open lead extending along the whole length of the mixer, is mounted between two hoppers, one arranged to deliver its contents into the mixer, and the second arranged to receive the contents from the mixer. A first gyratory sifter is arranged so that its outlet is connected to the inlet of the first hopper, and a second gyratory sifter is arranged to receive the outlet of the second hopper. The soya flour and ammonium chloride used in the additive are tipped directly into the first hopper and calcium sulphate is fed into this hopper through the first sifter. The contents of the first hopper are then transferred into the mixer, into which the enzyme preparation is introduced through the cover so as to be evenly distributed along the length of the mixer. The mixer is then operated in the desired length of time, after which it is stopped and opened, and the super glycerinated fat is added to the mixer in lumps which are introduced at evenly spaced distances along the whole length of the mixer. The mixer is then shut and mixing is continued for a further length of time. Its contents are then allowed to discharge into the second hopper and the mixed is then refilled in the manner stated above. The mixed additive is then slowly discharged from the second hopper, passed through the second sifter and bagged.

The invention may be performed in many ways, and will now be further explained by way of example.

Four different additives having the following compositions were made in the manner described above.

Ingredients %	No. 1	No. 2	No. 3	No. 4
Soya flour	94.4390	18.750	62.500	78.125
Fatty ester	1.3900	18.750	20.833	12.500
Calcium sulphate	2.6040	49.609	12.240	6.250
Proteolytic enzyme (Xn 18)	0.1770	0.391	0.261	0.196
Ammonium chloride	1.3890	12.500	4.166	2.929

The additives should be added to the flour in the following proportions.

No. 1. 9 lbs. per sack of 280 lbs;
No. 2. 2 lbs. „ „ „ „ „ ;
No. 3. 3 lbs. „ „ „ „ „ ;
No. 4. 2 lbs. „ „ „ „ „ ;

so that the final dosages of the various ingredients per sack of 280 lbs. in ounces are:

Ingedients oz. sack dosage.	No. 1.	No. 2.	No. 3.	No. 4.
Soya flour	136.00	6.00	30.00	25.00
Fatty ester	2.00	6.00	10.00	4.00
Calcium sulphate	3.75	15.875	5.875	2.00
Proteolytic enzyme	0.25	0.125	0.125	0.0625
Ammonium chloride	2.00	4.00	2.000	0.9375

It will be noticed that the proportion of proteolytic enzyme in composition No. 1 is lower than the proportion in composition Nos. 2 to 4, but that the dosage of enzyme per sack of flour in the case of composition No. 1 is double the dosage in the case of the other compositions. This is permissible in the case of composition No. 1, since the high dosage of soya flour tightens the dough and more proteolytic enzyme is therefore required to achieve the desired proteolytic effect on the dough. It should also be noticed that the proportion of ammonium chloride in composition No. 1 is lower than the proportion in the other compositions. However, as a result of the alteration in the dosage, the proportion of ammonium chloride per sack when using composition No. 1 is as great as when using composition No. 3 and more than twice the proportion when using composition No. 4. The fatty ester dosage of composition No. 1 is reduced since a large amount of fat (including lecithin) will be made available by the high proportion of soya flour. Finally, the calcium sulphate content of composition No. 1 is lower than that of the others, but the dosage per sack is comparable with that in composition Nos. 3 and 4. The sack dosages quoted above are suitable for ordinary bread doughs. In the case of Vienna Bread, composition No. 1 should be used at a dosage of 5 lbs. per bag of 140 lbs. of flour, No. 2 at a dosage of 2 lbs. per bag, No. 3 at a dosage of 3 lbs. per bag, and No. 4 at a dosage of 2 lbs. per bag.

Buns and similar leavened bakery are preferably made with the following composition, No. 5:—

No. 5.

Full fat soya flour	62.50%
Prepared fatty ester	18.75%
Ammonium chloride	6.25%
Proteolytic enzyme preparation (Strength 18 Xn units/gm)	0.39%
Calcium sulphate	12.11%

The prepared fatty acid ester has the following composition:

Free Fatty Acids	2.5%	Maximum
Soap	0.1%	„
Moisture	1.0%	„
Free Glycerol	4.0%	„
Mono-Stearate	40.45%	
Sap. Value	169—175	
Iodine „	56	
Melting Point	45°C.	

Such a fatty acid ester is sold by Advita Limited under the trade name "Rival".

The following Examples illustrate the uses of composition No. 5, which is the preferred additive for use when making bread or other leavened bakery.

EXAMPLE 1.

2 lbs. of the additive are added to 280 lbs. of wheat flour which is made into a dough. The dough is leavened and manipulated at 78°F. for three hours together with 5 lbs. of salt, 3 lbs. of yeast and water. The dough is then divided, moulded, proved and baked into bread in the conventional manner.

EXAMPLE 2.

2 lbs. of the additive are added to 140 lbs. of wheat flour which is made into a dough. The dough is fermented and manipulated for two hours at 74°F. together with 2¼ lbs. of salt, 2½ lbs. of yeast and water. The dough is then divided, moulded, proved and baked into fancy bread, and rolls in the usual way.

EXAMPLE 3.

2 ozs. of the additive are added to 4 lbs.—12 ozs. of wheat flour which is then made into a dough. The dough is fermented and manipulated for 1½ hours at 80°F. together with 6 ozs. of edible fat or oil, 8 ozs. of sugar, ¼ oz. of salt, 3 ozs. of yeast, and 2 pints of water. The dough is then divided, moulded, proved and baked as buns in the usual way.

The above compositions incorporate a proteolytic enzyme derived from Bacillus subtilis and having a strength of 18 Xn units per gram. When proteolytic enzymes having a lower activity, such as that derived from Aspergillus oryzae, are utilised, the following composition No. 6 is preferred.

No. 6.

Soya flour	62.50%
Fatty ester	18.75%
Calcium sulphate	11.63%
Proteolytic enzyme (Strength 4 Xn units/gm.)	0.87%
Ammonium chloride	6.25%

The dosage requirements for this composition are exactly the same as for composition No. 5, that is to say, 2 lbs. per sack of 280 lbs. of flour for bread, 2 lbs. per sack of 140 lbs. for Vienna Bread or fancy Bread, or 2 ozs. to 4 lbs. 12 ozs. of flour for buns or similar leavened bakery.

The fatty ester used in the above compositions Nos. 1 to 6 had the following characteristics:—

Free fatty acids	2.5%
Soap	0.1%
Moisture	1.0%
Free glycerol	4.0%
Glyceryl monostearate	40—45%
Saponification Value	169—175
Iodine Value	56
Melting Point	45°C.

For comparison purposes, the compositions of two conventional additives are given below:

Calcium sulphate	33%	33%
Ammonium chloride	12%	10%
Salt	24%	17%
Wheat flour	30.8%	17.8%
Potassium bromate	0.2%	0.2%
Glyceryl monostearate	—	22%

It is believed that mineral improvers con-

taining bromates, ammonium and calcium salts, and in some cases powdered glyceryl monostearate, depend for their effect on the "strengthening" action of the bromate on flour protein and on the activation of yeast by the ammonium and calcium salts. If powdered glyceryl monostearate is also present it is generally only employed in small quantities so that it does not have an optimum effect. In general the baker will usually prefer to add a glyceryl monostearate emulsion which he will prepare himself, together with a malt product to improve "gassing of the dough" and soya flour to improve the crumb, soya flour having a definite bleaching action on the crumb of the loaf. In the past, attempts to produce bread improvers incorporating soya flour have not met with great success, the baker generally preferring to add a rather larger quantity than can economically be incorporated into a conventional mineral improver. The present additive contains a sufficient proportion of soya flour to give the required bleaching action, together with the other effect mentioned above.

When conventional mineral improvers are employed, the crumb structure of the bread is made firmer and more resilient and staling of the crumb is retarded to some degree by the glyceryl monostearate. In contrast with using the present additive, it is found that the crumb structure is modified so that it becomes softer to the touch and the palate, while maintaining its natural resilience, and the staling of the crumb is retarded to a marked degree by the improvement in the action of the glyceryl monostearate. While it is believed that the incorporation of bromates in a mineral improver lessens the anti-staling properties of the fat, when the present additive is employed, the enzyme exerts a synergistic effect on the anti-staling activity of the glyceryl monostearate and the soya flour.

Furthermore, the present additive has the advantage over conventional additives that it renders the addition of malt products to increase the gassing rate of dough unnecessary. It also renders the dough more extensible and the action of the enzyme modifies the starches in the dough so that more sugar is rendered available for the nutrition of the yeast.

Normally, when the present additive is employed, fermentation proceeds more readily and as a result it is often possible to reduce the amount of yeast used.

The present additive may be used particularly advantageously when making buns, its use resulting in the formation of a "straight" dough, (that is to say a dough in which all the ingredients are mixed in one operation and which is then allowed to stand until it is ready for moulding) which rises as well as one made from a ferment, while the added softness of crumb and boldness of resulting bun, enables the baker to reduce the fat content by up to 25% and thereby to reduce the bun weight.

Vienna Bread and fancy bread, rolls, and the like can be prepared from a straight dough without the need for adding fat, sugar or milk powder. The crisp crust and the likely open crumb of this type of bread can therefore be obtained with the minimum of trouble and expense.

Finally, the present additive, unlike many conventional improvers, has a wide tolerance and may be used with all grades of flour in general use with equal success.

The amended "Rumsey" test, which is referred to above in this Specification, may be accomplished in the following manner. A glass dry stoppered 8 oz. bottle is suspended in a water bath, whose temperature is 27°C., for 30 minutes. 15 grammes of flour is placed in the bottle which is then returned to the bath for a further 20 minutes. 95 ml. of water at a temperature of 27°C. are then introduced into the bottle, which is shaken to form an even suspension of the flour in the water, and returned to the bath for one hour, the bottle being shaken at 15 minute intervals.

The bottle is then removed from the bath, 1.5 ml. of dilute sulphuric acid and 3.5 ml. of 15% sodium tungstate are added to, and mixed with, the suspension, and the suspension is then filtered through a number 4 Whatman filter, the first few drops which pass through the filter being returned to the solution which has not yet been filtered. The filtrate is titrated against 5 ml. of Fehlings No. 1 solution and 6 ml. of Fehlings No. 2 solution using the standard Lane & Eynon method. The sugar is estimated as maltose or referred to tables corrected for the method.

WHAT WE CLAIM IS:—

1. An additive for bread or other leavened bakery, which consists of, or includes, at least one fat or oil and at least one proteolytic enzyme derived from Bacillus subtilis or Aspergillus oryzae.

2. An additive as claimed in Claim 1, which also includes at least one phospholipid or phospholipid containing material.

3. An additive as claimed in Claim 2, in which the phospholipid containing material is soya flour.

4. An additive as claimed in Claim 2, in which the phospholipid containing material is wheat germ oil.

5. An additive as claimed in Claim 2, Claim 3 or Claim 4, in which the phospholipid containing material amounts to 15% to 95% by weight of the additive.

6. An additive as claimed in any one of the preceding Claims, in which the fat or oil consists of, or includes, a super-glycerinated fat or oil.

7. An additive as claimed in Claim 6, in

which the fat includes between 35% and 45% of glyceryl monostearate.

8. An additive as claimed in any one of the preceding Claims, which includes an ammonium salt, a calcium salt, or a mixture thereof.

9. An additive as claimed in Claim 8, in which the ammonium salt is ammonium chloride.

10. An additive as claimed in Claim 8 or Claim 9, in which the calcium salt is calcium sulphate.

11. An additive as claimed in any one of the preceding Claims, in which the activity of the enzyme is between 0.02 and 0.2 Xn unit per gramme of the additive.

12. An additive as claimed in Claim 11, in which the activity is 0.07 Xn unit per gram of the additive.

13. An additive as claimed in Claim 12, which contains 0.39% of a proteolytic enzyme preparation which has an activity of 18 Xn units per gram of the preparation.

14. An additive for bread or other leavened bakery substantially as described herein with reference to any one of the compositions Nos. 1 to 6.

15. A flour or dough containing an additive as claimed in any one of the preceding Claims.

16. Bread or other leavened bakery whenever prepared from a flour or dough as claimed in Claim 15.

17. Bread or other leavened bakery substantially as described herein with reference to composition No. 5 and any one of Examples 1 to 3.

18. A method of making bread and other leavened bakery, in which an additive according to any of Claims 1 to 14 is added to flour or dough before it is allowed to ferment.

19. A method of making bread substantially as described with reference to additive No. 5 and Examples 1, 2 and 3.

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PROVISIONAL SPECIFICATION

Improvements in or relating to Additives for Bread and other Fermented Products

We, WILLIAM LEONARD RUSSELL and WILFRED JOHN RUSSELL, British Subjects, of Albert Works, Chatsworth Road, Stratford, London, E.15, do hereby declare this invention to be described in the following statement:—

This invention relates to additives for bread and other fermented products and its object is to provide an additive capable of improving the fermentation process and thereby create a general improvement in the finished product. The invention is particularly concerned with the provision of a bread additive having the effect of keeping bread as fresh as possible, or in other words, of retarding staling of the bread. All such additives will hereinafter be referred to as "anti-staling" additives.

According to this invention there is provided an anti-staling additive having therein an enzyme possessing a controlled proteolytic effect. An enzyme of this kind should desirably only be used in minor proportions, that is in proportions below 1% and preferably below 0.5% based on an additive, normally employed in the proportion of 2 lbs. of additive per sack of 280 lbs. of flour. The most desirable proportion of enzyme appears to be 0.388% of the bread additive; that is, strictly speaking, a flour additive since it is added to the flour at an early stage in the breadmaking process, that is whilst the flour is being rotated in a conventional mixer and before the yeast and various liquids are added to the flour to form the dough.

The most efficient proteolytic enzyme at present available in this country is manufactured in two standard strengths, namely one containing 9% enzyme and the other 18% enzyme mixed with a suitable starch filler agent, and sold under the trade names of Proteinase 9 and Proteinase 18. Accordingly, it will be understood that the stated preferred percentage of 0.388% or 0.39% is concerned with a mixture which itself only contains 18% of active enzyme.

It is generally believed necessary to combine in the bread additive, with the proteolytic enzyme, relatively large proportions of ground leguminous seeds such as, in particular, soya flour, and also water-dispersible fatty esters such as various forms of partially esterified glycerol. A preferred material for this purpose is the commercial product glycerol monostearate which can be obtained in various concentrations and at various stage of purity. A preferred material of the fatty ester type for use with the enzyme appears generally to be a plastic or powder glycerol monostearate which requires no prior emulsification.

Certain mineral salts may also optionally be used and may also have beneficial effects on the anti-staling additive. Of these salts, the preferred materials are calcium sulphate and ammonium chloride, and the total mineral salts should desirably be approximately equal to the proportion of the fatty ester employed, e.g. the glycerol monostearate. Provided highly oxidising salts such as bromates and persul-

phates are avoided, most of the mineral salts and similar additives normally employed may also be used. In general, however, these mineral salts should not be employed in very high proportions relatively to the soya or equivalent flour content in the additive. Ascorbic acid may also be used although only in very small proportions.

In general, as regards the mineral salt content, ammonium salts are the most desirable ingredients, and of these salts, ammonium chloride is that generally employed. However, calcium and ammonium phosphates and calcium sulphate are also suitable ingredients, preferably in combination with the ammonium chloride. Of the normal sugar additives, sucrose, invert sugar, and lactose may be used, but malt products are in general undesirable since they lead to a highly sticky dough and/or crumb. Of the fats generally, lard and hydrogenated shortenings may be used, but these materials do not appear as useful as the fatty esters such as glycerol monostearate referred to above. For instance, it has been found that using the commercial grade of glycerol monostearate, which is about 35% monostearate, at the rate of 4 ounces per sack of flour, a marked anti-staling effect is obtained. Materials generally known as P.O.E.M.S. and T.E.M. have a similar action at lower concentrations. However, these fats have to be emulsified with hot water before they can be used, which often leads to practical difficulties; in addition, they should not be employed in excess since they would otherwise lead to an early breakdown of the crumb of the loaf. The material Abracol W which is a coated type of synthetic fat is dispersible in cold water and may therefore be employed, although it does not form an emulsion as such.

Of the commonly used milk products, skimmed milk powder and whey powder may also be used; the latter material has objection that it may impart an undesirable flavour to the loaf and it has a tendency to cake on storage. It should be noted that the soya flour mentioned above as a valuable ingredient forming preferably a large proportion of the additive, has a high fat and protein content, and is biologically similar to milk powder so that it constitutes as such a useful source of protein for released organisms.

Before considering in greater detail the various properties of the ingredients suggested above, and the possible action of the most essential ingredients in giving the "anti-staling" effect of the invention, various tests will now be indicated including a comparison between three forms of additives which has led to the discovery of what appears to be the most effective anti-staling additive so far developed.

Eight compositions were compared and were made up as follows:—

1. The control composition without any anti-staling additive.
2. Composition 20258.
3. Seftex, which is a conventional fat-type improver used in conjunction with mineral improvers.
4. Seftex plus Potavie M.
5. Bacterase (0.4 grams per 71 lbs.)
6. Proteinase 18 (0.2 grams per 71 lbs.)
7. Composition 20258 (control for Test 8).
8. Composition 20258 plus 1 lb. of soya per sack of flour.

Test 1 gave an "attractive" dough which moved well at all stages. The bread was of good volume with a nice colour, a firm crumb, and a good sheen.

Test 2 gave a dough which was slower moving than that of Test 1, but this was thought to be due to its tighter springy quality. The bread gave a "bold" loaf with a good colour in the crust and crumb, although the latter was inclined to be dull.

Test 3 gave a dough similar to that of Test 1, but a little softer to handle. In texture, the dough was similar to that of Test 2, as was the bread when cut. The colour of the crumb was better than that of Test 2.

Test 4 gave a dough which moved steadily and a shade faster than that of the previous tests, which handled well and was generally similar to that of Test 2. The resulting bread was a little open in texture, and of rather large volume. For this reason, the crumb colour did not appear very satisfactory.

Test 5 gave a very sticky dough, and this was reflected in the finished bread by a complete collapse in the crumb at the slightest pressure, coupled with an open, blown texture.

The dough of Test 6 was also slightly soft and sticky, but very much less than that of Test 5. A little trouble was experienced in moulding dry. The bread was slightly loose in the crumb and open in texture, but recovered well after pressure. In colour, the crumb was very similar to the control (Test 1).

Test 7 was, of course, similar to Test 2, although by slight alteration in composition to conform with Test 8, the crumb had a closer texture and a better colour.

Test 8 gave a slightly tighter dough than Test 7, and the resulting bread was very similar in texture and crumb colour to that of Test 7.

Crumb compression tests were carried out on the above eight compositions using an apparatus based on the standard compression-testing B.B.I.R.A. instrument with certain modifications to simplify construction and to improve calibration. Owing to the rapidity with with the baking was carried out, it was not possible to carry out compression tests until the bread from Tests 1 to 6 was six days old, and that from Tests 7 and 8 four days old. Each compression test took four

minutes to carry out using two operators, and the samples had, of course, to be cut to a definite thickness and size just prior to the test. Three slices were taken from each loaf, and two loaves from each batch; approximately eight hours were spent on the entire series. Owing to the instrument and method employed, the tests were only of an approximate nature, but a definite trend could be extracted from the results obtained. Thus, composition 20258 showed an improvement in keeping quality over the control, whilst Seftex did not. The bacterase bread of Test 5 collapsed under the test and did not recover, whilst the proteolytic enzyme bread (Test 6) gave similar compression results to the Seftex bread. Seftex plus Potavic M (Test 4) was similar in its action to composition 20258 and slightly better in keeping quality. The bread from Tests 7 and 8 was very similar, and in this case the soya flour apparently did not improve the keeping quality beyond any improvement obtained from composition 20258 alone.

Gassing tests were carried out for total and retained gas on certain of the doughs, and for retained gas alone on all the doughs. Tests 3, 5, 7 and 8 all showed about the same total gas production, whilst Test 6 (proteolytic enzyme) showed an increase in gas production, although the extra gas was not retained and did not give an increased volume in the dough.

The conclusion from these preliminary tests was quite obviously that Bacterase was not suitable for use as an anti-staling agent in bread, whilst proteolytic enzyme had a marked although not extreme effect on the dough and on gas production. It was also superior to composition 20258 which contained Amylosyme B as its active ingredient. The results obtained with Seftex, 20258 and Seftex plus Potavic M confirmed previous observations.

It was found from the experiments that bromate could not be used in the improved additives as its action was apparently contrary to that of proteolytic enzyme.

Including control doughs, 25 further tests were carried out to study the effects of basic ingredients and combinations of additives as bread improvers and crumb softeners.

A mixture of four parts of soya flour and one part of plasticised G.M.S. did not materially affect the rate of fermentation, but a definite improvement in keeping qualities was obtained. 1 lb. per sack appeared to be sufficient, as double this amount did not effect further improvement. 1 lb. of this mixture, plus 1 lb. of 20258 per sack improved for fermentation and loaf volume, but did not appear to impart a further improvement in crumb softness. The crumb colour of this set of loaves was an improvement over that of the control. The following mixtures were prepared in accordance with the stated proportions:—

Additive	17358A.	17358B.	20358.
Soya Flour	20.0	20.0	20.0
"Rival" G.M.S.	6.0	6.0	6.0
Ammonium Chloride	2.0	2.0	2.0
Gypsum	3.375	—	3.875
Lactose	—	3.375	—
Proteinase 18	0.125	0.124	0.125
S.F.P. Product	0.500	0.500	—

The various numbers referred to above refer to ounces per sack of 280 lbs. of flour, each additive comprising 2 lbs. of total ingredients. The S.F.P. Product was a material sold under the trade name of Amylosyme B, which contained the active amylase ingredients in a starch carrier. Additives 17358A and 17358B were each tried at 1 lb. and 2 lbs. per sack, and were compared for effect with Super Potavie employed at the rate of 1 lb. per sack.

At the rate of 1 lb. per sack, the new additives did not appear to have a marked advantage over Super Potavie, but at 2 lbs. per sack the difference was quite definite, especially over a 96 hour period when the loaves with the new additives, that is additives 17358A and B and additive 20358, were almost as fresh as the control loaves after 24 hours. The additive 20358 was found to be the preferred composition, but was similar to 17358A, which latter additive was slightly

superior to 17358B. In general, it was found, as a major conclusion, that the S.F.P. Product was unnecessary and possibly undesirable and therefore should be omitted.

All these additives improved the fermentation and total gas produced, although the Super Potavie doughs were somewhat more resilient at the scaling stage. A slight stickiness was observed in all the doughs which were made up with 16 gallons of water per sack. All the additives showed improved loaf volume and crumb colour as compared with the conventional additives, such as Super Potavie, additive 20258, Seftex and so on.

Composition 20258 contained two parts of ammonium chloride, one part of calcium sulphate, 12 parts of G.M.S. powder which was dispersible in cold water, 0.3 part of bromate, and one part of amylosyme B. Potavie and Super Potavie were commercial gluten-stabilised yeast foods containing mineral salts, in particular bromates. Bacterase was a commercial amylase material of bacterial origin which had been suggested as a suitable test material for breaking down the envelope of the starch cells in the flour.

The conclusion of these latter tests was that additive 20358 (containing the active proteolytic enzyme) had a definite action on the bread dough leading to improved fermentation. The crumb of the bread produced from these doughs was pleasing in texture, was of improved colour and softness and had a marked resistance to staling.

Considering now the general theory on which the above conclusions and investigations were based, the object of testing the various additives for bread and similar fermented goods, was to improve the fermentation processes and thereby to create a general improvement in the finished product. The direction of this improvement was in the sense of obtaining a bread which would keep for a substantially longer period than the conventional loaves of bread. This object was considered of great importance in view of the present prevalence of sliced wrapped loaves which have a tendency to stale on opening of the wrapping, or even after lengthy transport and storage within the wrapper.

It is believed that the desired direction of the improvement is brought about by enzymic action of the proteolytic agent on the envelopes of the starch cells, which action causes a controlled breakdown of the cells and affects them in such a way as to cause the absorption of more moisture in the finished baked products. It is for this reason that reference was made above to the use of an enzyme having a controlled proteolytic effect as opposed, for instance, to the normal proteolytic enzymes in the natural wheat which are normally removed at the milling stage since they would cause so pronounced a brakedown of the starch as adversely to affect the finished product.

As indicated above, it is believed at present that water-dispersible esters such as glycerol monostearates, which esters should preferably be enhanced by leguminous flour such as soya flour, are, if not essential, at least highly desirable so as to have a barrier effect on the water absorbed due to the enzymic action on the starch cells. Thus, the fatty materials, due to their surface tension effects, will prevent the water which has been absorbed from evaporating out of the cells and will therefore have a "holding" action on the water. Part of the theory underlining the use of the present proteolytic enzyme additives is the suggestion that the starch cells do not, as was commonly thought, have cellulosic envelopes, but possess proteineous envelopes. The choice of enzymes therefore allows controlled breakdown of these envelopes by proteolytic action which allows more water to enter into the interior of the cells and stimulates fermentation by causing more sugar to be available. It should be noted in any case, that proteolytic enzyme type material will enhance the fermentation action of the yeast so that the additive may well have a double effect on the fermentation process. As indicated above, the enhanced fermentation is disclosed by the increased production of gas found with proteolytic enzyme additives. For the desired result, it is also important that, after cooling, the barrier materials, such as the soya-enhanced G.M.S. mentioned above, should prevent the loss of moisture which is absorbed in the starch cells.

The use of ammonium salts such as ammonium chloride, is suggested by the fact that such materials act as yeast stimulants in supplying the essential ammonium group for reproduction and formation of healthy cells, which are thus able to use the sugars produced in the dough during the final proof stage to best advantage. Phosphates may also be used for this purpose, but the naturally occurring phosphates in the flour are generally sufficient for normal yeast metabolism. It is doubtful whether the invert sugar is of importance as it would seem that the yeast will naturally utilise any added amount of this material at an early stage in fermentation. The lactose which may be employed caramelises at a fairly low temperature and hence imparts a high colour and nice bloom to the crust of the loaf. As regards malt products, their proteolytic activity is such that they have a marked adverse effect on dough stability so that they are generally undesirable. Since alpha amylase is always present in amounts greater than is required, the danger of a sticky crumb is very real when malt products are used.

The G.M.S. effect is believed to be due to its ability to combine with the natural fat in the flour and form a "barrier" in the crumb

of the loaf preventing water loss from the starch through syneresis. P.O.E.M.S. and T.E.M. have a similar action at lower concentrations. The use of any of these products in excess leads to early breakdown in the crumb of the loaf after cooling.

As regards the soya flour, this has a high fat and protein content and the fat portion includes a high proportion of unsaturated oils with about 2% of lecithin, a natural emulsifier. The small amount of saturated fatty acids is believed to assist in strengthening the gluten of the dough, and in making it more elastic. The total effect is to give a sticky crumb with improved softness, high water absorption and therefore increased yield. In some cases a slight bleaching effect is also noticed. As regards the various amylases which have been employed for comparative purposes, these may be of fungal and of bacterial origin. The Bacterase is apparently a starch-liquefying enzyme preparation which will convert gelatinised starches to soluble starches, and if the process is continued long enough to dextrins and sugars. It is clear that this effect is far too strong for the purposes of the invention. The action of these enzymes in common with cereal enzymes depends to a large extent on the amount of available starch present in the dough. Accordingly, it seems that the addition of alpha amylase does not make any more "raw" starch available but merely pushes up the total conversion of the available starch to dextrine and sugars carried out by beta amylase. Thus, in a dough containing a sufficiency of cereal alpha amylase, there will be little, if any, increase in gas production due to an increase in available sugars. On a three hour process the yeast is fed mainly by the original sugars present in the flour. Only in the latter part of the proof stage and early part of the baking does the enzyme-converted starch play an important part. The effect on gas production of the maltase content of amylosyme would therefore appear to be unimportant in a yeasted dough, as the yeast organism apparently secretes this enzyme in exactly the right amount required for its metabolism. In conclusion, as regards the amylases, the increase in total sugars and dextrined starches will no doubt improve the moistness of the crumb, the crust colour, the loaf flavour, and the toasting characteristics. On the other hand, the flour with a sufficiently high level of cereal alpha amylase will give similar results and so mask the effect of additional fungal amylase. When comparing loaves from doughs containing malt, soya and so on, with loaves from doughs containing only amylase, these remarks will apply even more strongly.

As regards the Proteases, these act on the flour protein, that is the gluten of a dough is apparently softened by their action and made more extensible. Malt products contain enzymes of this type and for that reason doughs made with these products tend to soften quickly if a slight excess is used.

In final conclusion, it should be noted that the formulation of bread improvers in the past has been based on a mixture of gluten stabilisers and yeast activators coupled with the addition of powdered G.M.S. which may or may not be dispersible in cold water. The introduction of tablet and packet "dosage" improvers has led to the separation of fat and mineral additives, and in the case of "Sefcol" to the addition of fungal enzymes to the "mineral" improver. "Seftex" was a "fatty type" improver used in conjunction with mineral improvers as mentioned above.

The present additive such as additive 20358, was formulated to couple various types of improver into one additive since past experiments have failed in general to show any increase in the gassing rate of fermentation of a dough. Attempts were made in the direction of gas improvement and once such gas improvement had been obtained, attempts were made effectively to "seal" the water into the dough as much as possible so as to assist in the fermentation. It is believed that the protease acts in a direct manner by improving fermentation, both by easing the access of water to the interior of the cells and by enhancing yeast behaviour whilst the G.M.S. and soya type components act in a passive manner. Thus, they maintain the improvement due to the protease component by retaining the water as much as possible.

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